U.S. Army Medical Research
Institute of Chemical Defense

USAMRICD-TR-07-05

Stability of Dilute Tabun (GA) Solutions in Various Vehicles

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November 2007

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REPORT DOCUMENTATION PAGE

1. REPORT DATE (DD-MM-YYYY)	2. REPORT TYPE	3. DATES COVERED (From - To)
November 2007	Technical Report	

4. TITLE AND SUBTITLE
Stability of Dilute Tabun (GA) Solutions in Various Vehicles

5a. CONTRACT NUMBER

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Logan , TP, Swift , AT, Way, MR, Lyles, JJ, Soni, S-D

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

US Army Medical Research Institute of Chemical Defense
ATTN: MCMR-CDT-D
3100 Ricketts Point Road
Aberdeen Proving Ground, MD 21010-5400

8. PERFORMING ORGANIZATION REPORT NUMBER

USAMRICD-TR-07-05

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
US Army Medical Research Institute of Chemical Defense
ATTN: MCMR-CDZ-P
3100 Ricketts Point Road
Aberdeen Proving Ground, MD 21010-5400

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for public release; distribution unlimited

13. SUPPLEMENTARY NOTES
This research was supported by the Defense Threat Reduction Agency – Joint Science and Technology Office, Medical S&T Division.

14. ABSTRACT

Tabun (GA) is unstable in aqueous media, which has created difficulties in supplying reliable preparations of GA in saline for toxicity studies. We previously demonstrated the stability of GA in Multisol (M), a four-component solvent (48.5% H_2O, 40% propylene glycol, 10% ethanol, and 1.5% benzyl alcohol) used in various chemical preparations. However, M's 10% ethanol content may alter toxicity findings. We used ^{31}P nuclear magnetic resonance (P-NMR) and gas chromatography mass spectrometry (GCMS) to investigate the stability of GA in two modifications of M. Mixture #1 contained 48.5% D_2O, 45% propylene glycol, 5% ethanol, and 1.5% benzyl alcohol; #2 contained 48.5% D_2O, 50% propylene glycol, and 1.5% benzyl alcohol. Using P-NMR we found GA to be stable for approximately 3 h at 25 °C in both mixtures. Using GCMS we found that GA was stable in both mixtures at -70 °C for more than 14 months. We performed separate dilutions of GA in mixture #1, mixture #2, and the original M with saline prepared with D_2O; we monitored GA's stability. Using P-NMR we demonstrated GA's stability at 25 °C for approximately 9 h in the dilutions of both mixtures and for at least 3 h in the original M dilution. This work demonstrated that the reduction or removal of ethanol from M can be accomplished without unduly influencing GA's stability for researchers and provides the length of time of GA's stability when diluting M vehicles with saline prepared with D_2O at 25 °C. These vehicle modifications may be helpful in future GA toxicity studies.

15. SUBJECT TERMS

GA, Tabun, Multisol, Nuclear Magnetic Resonance, Gas Chromatography, Mass Spectrometry, Stability

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT UNCLASSIFIED	**b. ABSTRACT** UNCLASSIFIED	**c. THIS PAGE** UNCLASSIFIED	UNLIMITED	15	Thomas P. Logan
					19b. TELEPHONE NUMBER (include area code) 410-436-1484

Abstract

Tabun (GA) is unstable in aqueous media. This instability has created difficulties in supplying reliable preparations of GA in saline for toxicity studies by our researchers. Multisol (M) is a four-component solvent (48.5% H_2O, 40% propylene glycol, 10% ethanol, and 1.5% benzyl alcohol) used to prepare various chemical preparations. We previously demonstrated the stability of GA in M. However, M's 10% ethanol content may alter toxicity findings when using it to investigate the toxicity of agents. We used ^{31}P nuclear magnetic resonance (P-NMR) and gas chromatography mass spectrometry (GCMS) to investigate the stability of GA in two modifications of M. Mixture #1 contained 48.5% D_2O, 45% propylene glycol, 5% ethanol, and 1.5% benzyl alcohol, and #2 contained 48.5% D_2O, 50% propylene glycol, and 1.5% benzyl alcohol. Using P-NMR we found GA to be stable for approximately 3 h at 25 $^\circ$C in mixtures #1 and #2. Using GCMS we found that GA was stable in mixtures #1 and #2 at -70 $^\circ$C for more than 14 months. Furthermore, we performed separate dilutions of GA (1.9 mg/ml to 0.24 mg/ml) in mixture #1, in mixture #2, and in the original M with saline prepared with D_2O, and we monitored GA's stability. Using P-NMR we demonstrated GA's stability at 25 $^\circ$C for approximately 9 h in the dilutions of mixture #1 and mixture #2, and for at least 3 h in the original M dilution. This work demonstrated that the reduction or removal of ethanol from M can be accomplished without unduly influencing GA's stability for researchers and provides the length of time of GA's stability when diluting M vehicles with saline prepared with D_2O at 25 $^\circ$C. We concluded that these vehicle modifications may be helpful in future GA toxicity studies.

Introduction

Tabun's (GA) instability in aqueous media is a problem when preparing saline solutions of this analyte. GA's structure is highly susceptible to hydrolysis at the -CN, and -N(CH$_3$)$_2$ bonds to phosphorus.
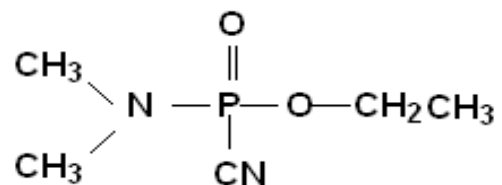
In neutral or near neutral aqueous solutions, GA hyrolyzes rapidly at the P-CN bond, yielding ethyl hydrogen N,N-dimethylphosphoroamidate as the major product.[1,2] It has been our personal experience and the experience of others[3] that the nerve agents sarin (GB), soman (GD), and cyclosarin (GF) in saline are stable for months when stored at -70 °C. However, GA in saline solutions decomposes after several weeks at this temperature.

Multisol (M) (48.5% H$_2$O, 40% propylene glycol, 10% ethanol, and 1.5% benzyl alcohol) has been used to solubilize compounds that were found to be relatively insoluble in saline.[4-8] The composition of M is similar to the vehicle used with injectible Valium™.[4] Other workers found that M provided stable GA solutions.[3,9,10] However, the 10% ethanol component in M concerns researchers in that it may alter toxicity findings for an analyte in a solution of M. We therefore evaluated changes in M's composition, first reducing and then removing ethanol. We chose ^{31}P nuclear magnetic resonance (P-NMR) to investigate the stability of GA at 25 °C in two modifications of M, referred to as mixture #1 (m#1) and mixture #2 (m#2). The ethanol content was reduced to 5% in m#1 and removed completely in m#2; H$_2$O was replaced with D$_2$O in both mixtures. In each mixture we increased propylene glycol to compensate for ethanol changes. We then investigated the stability of GA in m#1 and m#2 after storage at -70 °C for 14 months using gas chromatography mass spectrometry (GCMS).

Another GA stability question arises when researchers further dilute the solutions of GA in M with saline for toxicity studies since they then need to know how long GA is stable in the diluted solutions. P-NMR was again used to investigate the stability of GA in M, m#1, and m#2 after each of these three solutions was diluted with saline prepared with D$_2$O.

Materials and Methods

Materials. Tabun (Dimethyphosphoramidocyanidic acid, ethyl ester, GA) was obtained from the US Army Edgewood Chemical Biological Center (ECBC), Aberdeen Proving Ground, MD; GA purity was 98.5% as determined by NMR spectroscopy. Deuterium oxide 99.9 atom % D and acetonitrile (CH$_3$CN), HPLC grade were obtained from Aldrich Chemical Company, Milwaukee, WI. Absolute alcohol was obtained from Pharmco, Brookfield, CT. Propylene glycol was obtained from Phoenix Pharmaceutical, Inc., St. Joseph, MO. Benzyl alcohol was

obtained from Eastman Kodak, Rochester, NY. Chloroform ($CHCl_3$), high purity solvent, was obtained from Burdick and Jackson, Muskegon, MI. Diisopropyl methanephosphonate (DIMP) was obtained from Lancaster Synthesis, Pelham, NH.

P-NMR measurements were made of GA in m#1, GA in m#2, and separate dilutions of GA in M, m#1, and m#2 made with D_2O saline. Solutions of GA in M, m#1, and m#2 were prepared gravimetrically at 1.9 mg/ml with D_2O substituted for H_2O and stored at -70 °C. Immediately upon thawing and mixing at room temperature, 700 uL of the solution was transferred into a 5 mm o.d. NMR tube. P-NMR measurements were made of 0.24 mg/ml GA solutions prepared by mixing 88 uL of the gravimetrically prepared solutions of GA with 612 uL of D_2O saline in an NMR tube. All NMR data was collected on Varian Unity Inova spectrometer equipped with a 5 mm gradient Penta[R] probe. Probe temperature was maintained at 25 °C $\pm$ 0.1 °C, and the VT air flow was maintained at 10 LPM. Standard s2pul sequence with continuous proton decoupling [$^{31}P\{^{1}H\}$] was used, and all the calibrations were done as per the installation procedure. Chemical shifts are reported in ppm (parts per million) using 85% phosphoric acid (H_3PO_4) as external reference at -0.9 ppm.

Gas chromatographic analyses of GA in M, GA in m#1, GA in m#2, and $CHCl_3$ were made after 1:100 dilution with CH_3CN containing 0.014 mM DIMP as the internal standard. An Agilent 6890 gas chromatograph interfaced with a 5973 mass selective detector was used with electron energy set to 70 electron volts. GA was analyzed in the selected ion monitoring (SIM) mode using the three principal ions of GA (*m/z* 70, 133, 162) and the three principal ions of DIMP (*m/z* 97, 123, 79) using dwell times of 30 microseconds for each ion. The gas chromatograph was equipped with a 30 m, 0.25 mm i.d., 0.25 mm HP-5MS column. The injection port was at 250 °C, and the initial oven temperature was 70 °C for 2 min, and the temperature ramp rate was 30 °C min^{-1} until the final temperature of 250 °C was reached and held for 3 min. Samples were run in the splitless mode with a helium carrier gas flow rate of 1 ml min^{-1} measured at 70 °C.

Results

P-NMR was used to investigate the stability of GA in two modifications of M (m#1 and m#2) at 25 °C. Figure 1 shows the stability of GA in m#1 over a 13-h period in a sample of GA prepared at 1.9 mg/ml and using D_2O in place of H_2O. The -10 ppm peak is the GA resonance,[2] and it remained the major component throughout the run. Small resonances that do not increase in size are impurities. Small resonances appeared and began to grow in size after 3.5 h. We attributed these resonances to P containing decomposition products of GA. Figure 2 follows the stability of GA in m#2 over a 13-h period in a sample of GA at 1.89 mg/ml and using D_2O in place of H_2O. Again the GA resonance at -10 ppm peak remained the major component after 13 h. Decomposition appeared to be under way at the 5-h point.
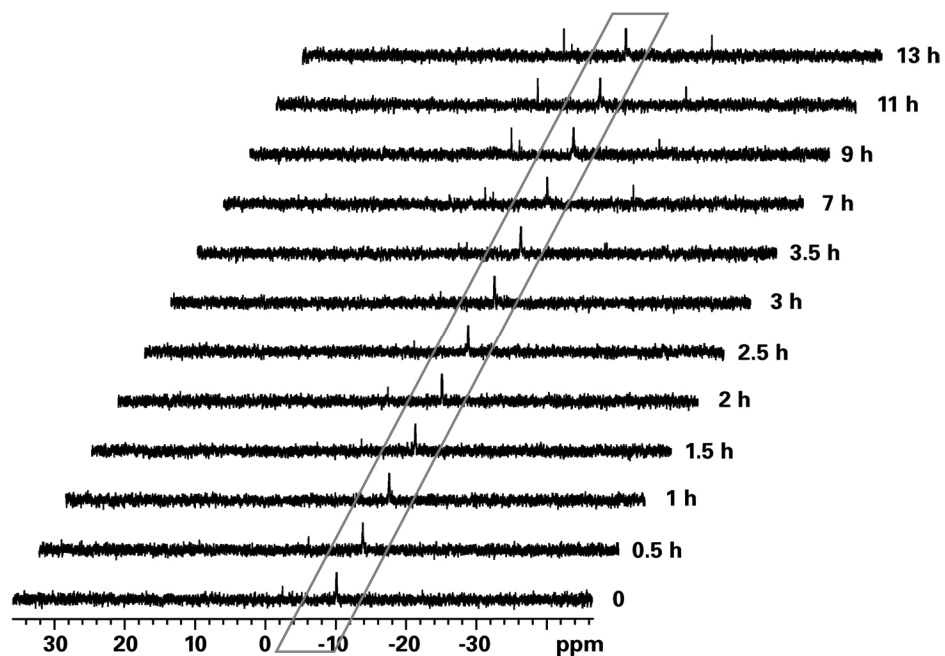
Figure 1

Figure 1 followed the stability of GA in m#1 over a 13-h period at 25 °C using P-NMR to monitor the GA and decomposition product resonances in a sample of GA at 1.9 mg/ml. The GA resonance at -10 ppm[2] remained the major component for 13 h. Small resonances that proceed to grow in size occurred at 3.5 h. We attributed these resonances to P containing decomposition products of GA.
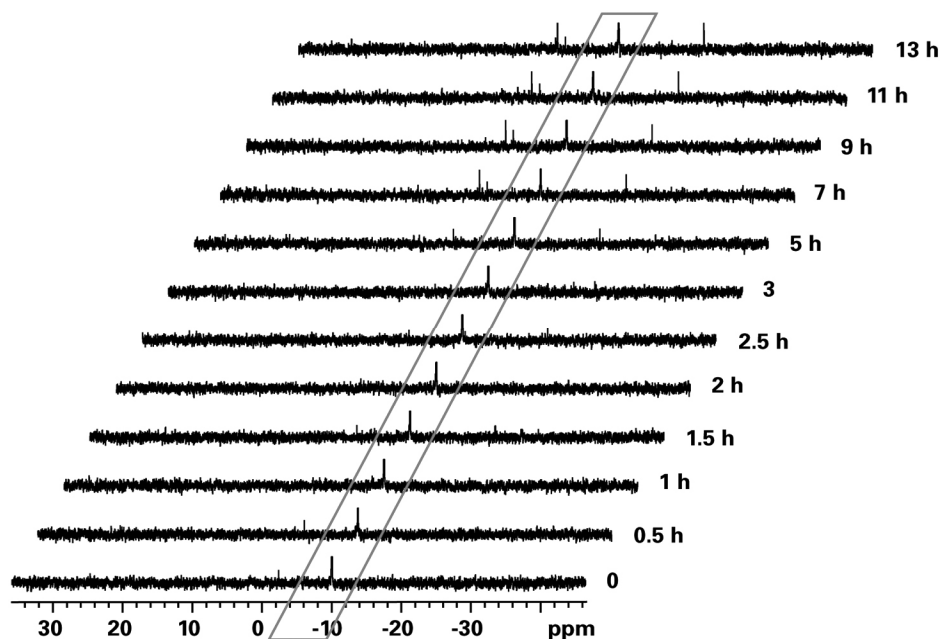
Figure 2

Figure 2 followed the stability of GA in m#2 over a 13-h period at 25 °C using P-NMR to monitor the GA and decomposition product resonances in a sample of GA at 1.9 mg/ml. The GA resonance at -10 ppm^2 remained the major component for 13 h. Small resonances that proceed to grow in size occurred at 5 h. We attributed these resonances to P containing decomposition products.

The stability of diluted solutions of GA in M, m#1, and m#2 with D_2O saline is shown in Figures 3, 4, and 5. We did not detect evidence of GA decomposition for 9 h after diluting m#1 with D_2O saline (Figure 3). Similarly, we did not detect evidence of GA decomposition until the 9-h time point after diluting m#2 with D_2O saline (Figure 4). Finally we did not detect GA decomposition in the comparatively short time of 3 h after diluting M with D_2O saline (Figure 5).

Previously, we studied the stability of 1.9 mg/ml solutions of GA in M after storage at -70 °C. In this study, preparations of GA in M, m#1, and m#2 using D_2O to replace H_2O were stored for more than 14 months at -70 °C. We chose GCMS to analyze these solutions using GA in $CHCl_3$ as a reference standard. Figures 6A and 6B are typical chromatograms of standard and sample m#2 used for the results in Table 1.
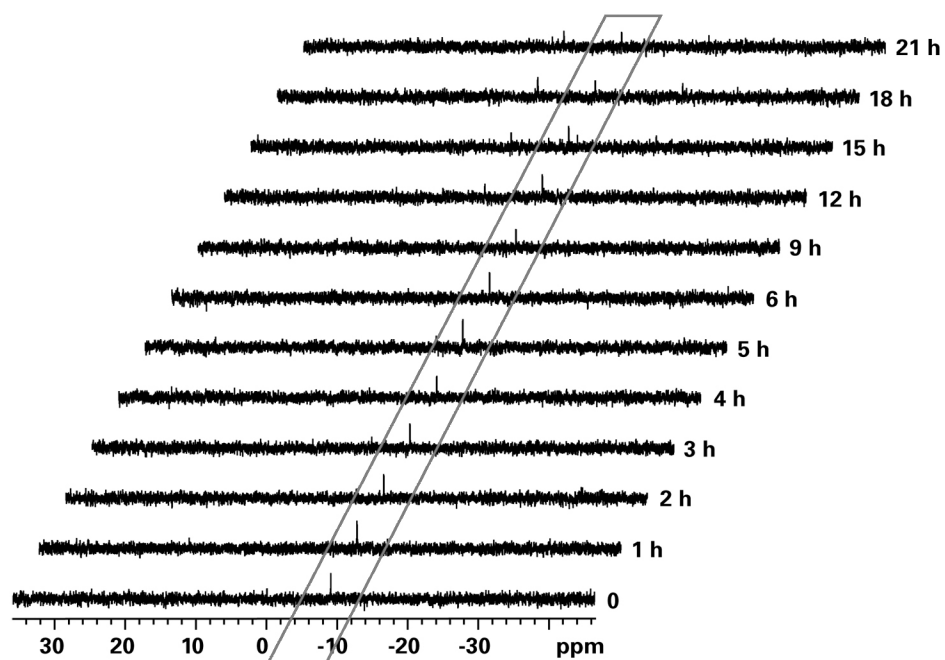
Figure 3

Figure 3 shows the stability of GA in a diluted sample for 21 h at 25 °C using P-NMR to monitor the GA and decomposition product resonances. The 0.24 mg/ml sample was prepared by mixing in GA in m#1 with D_2O saline. We did not detect evidence of GA decomposition for 9 h.
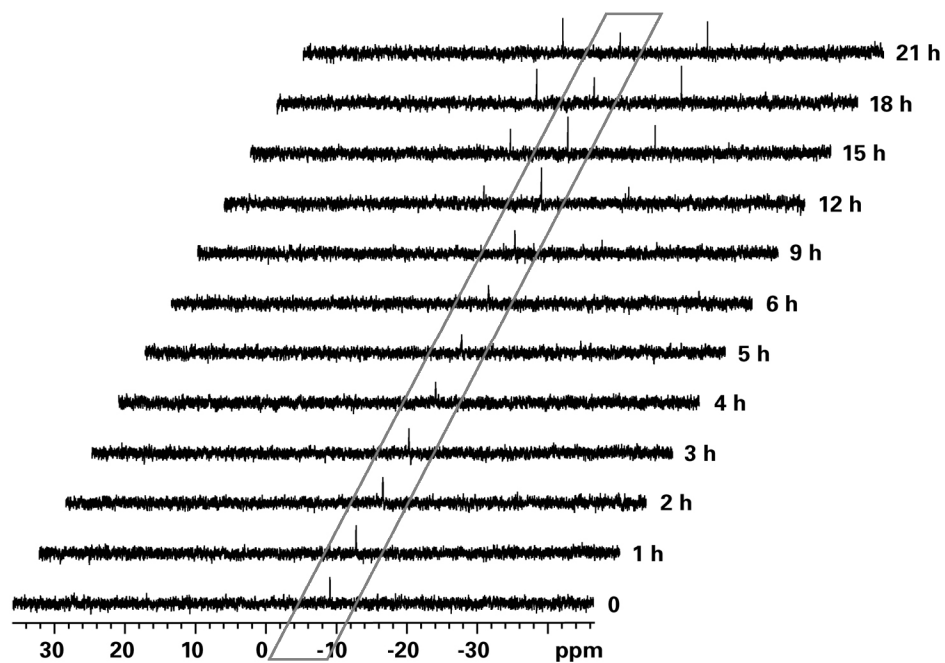
Figure 4

Figure 4 shows the stability of GA in a diluted sample for 21 h at 25 °C using P-NMR to monitor the GA and decomposition product resonances. The 0.24 mg/ml sample was prepared by mixing in GA in m#2 with D_2O saline. We saw evidence of GA decomposition at 9 h.
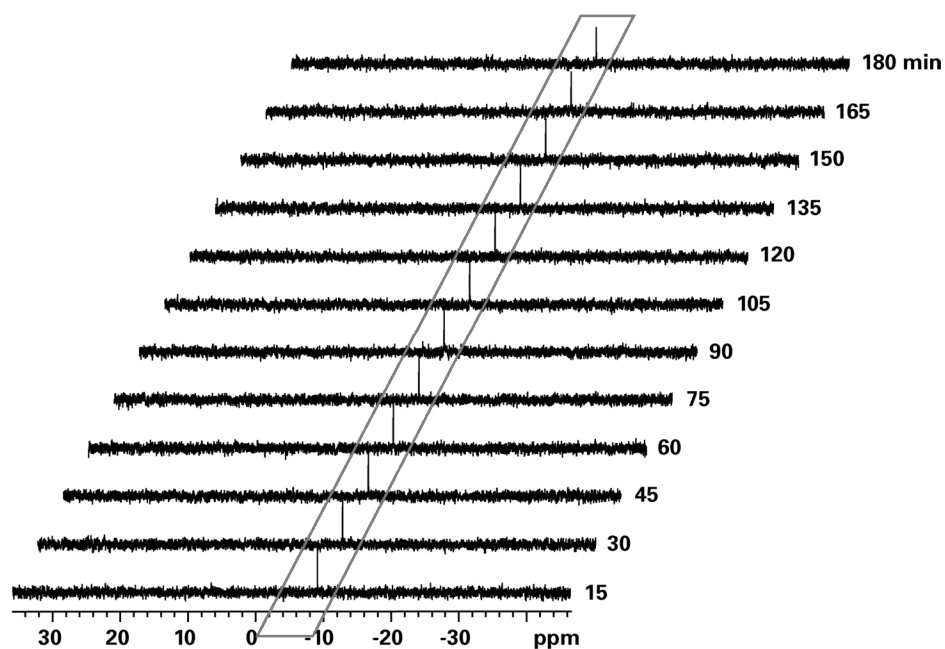
Figure 5

Figure 5 shows the stability of GA in a diluted sample for 3 h at 25 °C using P-NMR to monitor the GA and decomposition product resonances. The 0.24 mg/ml sample was prepared by mixing in GA in M with D_2O saline. GA appeared stable during this 3-h period.
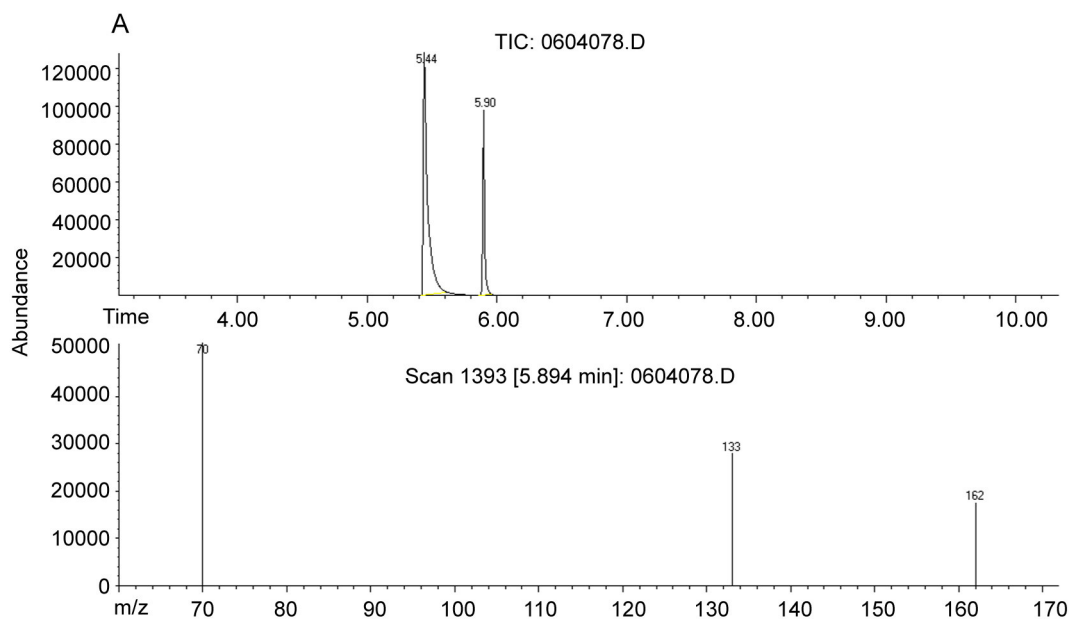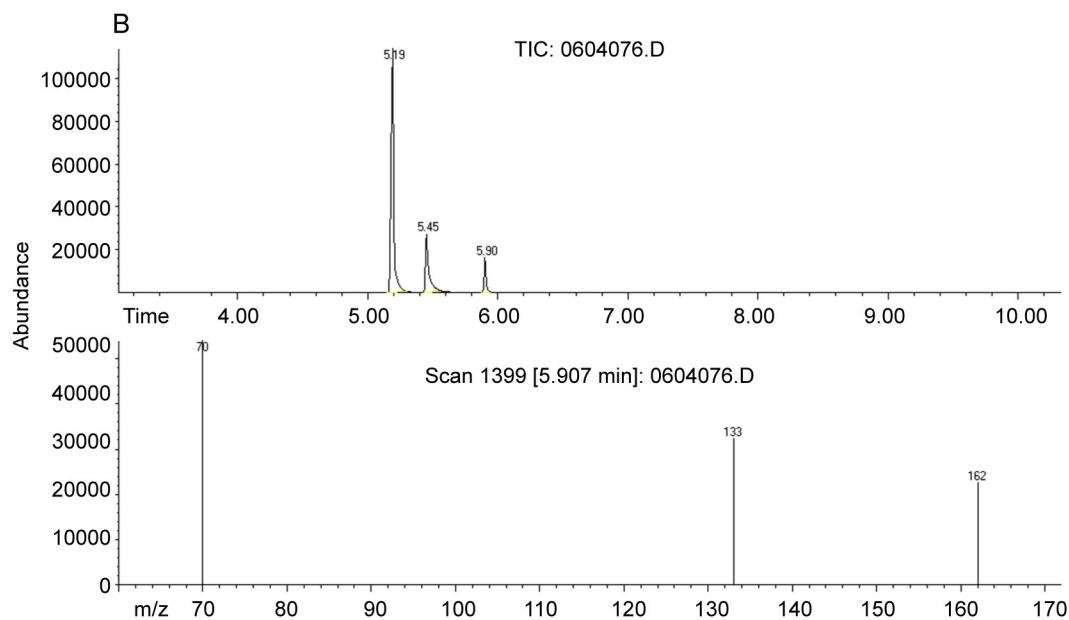
Figure 6A

Figure 6B

Figures 6A and 6B are GCMS chromatograms of GA in CHCl$_3$ and GA in m#2 respectively. Figure 6A is one of the reference chromatograms used for the calculation of GA's concentration in M, m#1, and m#2. The peak at 5.44 min is DIMP and at 5.90 min is GA. The large first peak in Figure 6B is benzyl alcohol.

Table 1

GA solution and preparation date (ddmmyy)	Gravimetric preparation Concentration mg/ml	GCMS Analysis 060407 Concentration mg/ml {%Coefficient of Variation, [CV], n = 3}
GA in M 081205	1.87	1.94 (5.1%)
GA in m#1 180106	1.90	1.86 (3.6%)
GA in m#2 180106	1.89	1.97 (1.8%)
GA in CHCl$_3$ 090905	1.89	reference standard

Table 1 contains the GCMS analysis results of GA in three solvent vehicles after more than 14 months of storage at -70 °C, demonstrating GA's stability in M, m#1, or m#2.

Discussion

The results of this study demonstrated that GA (1.9 mg/ml) was stable for approximately 3 h at 25 °C in m#1 and in m#2. We previously[10] demonstrated GA's stability for 14 h in M and less than 1 h in D_2O saline. Furthermore, we can reduce or remove ethanol's influence in GA toxicity studies by using m#1 and m#2. Although GA's stability at 25 °C was reduced to 3 h in these two mixtures from 14 h in M, the researcher still has ample time for its use in toxicity studies at 25 °C.

The GCMS analysis indicates that GA has stability in m#1 and m#2 for more than 14 months when stored at -70 °C. This is similar to the stability of GA in M at -70 °C previously reported,[10] and that is a large improvement compared to GA's stability of weeks in saline solution. It should be noted that M, m#1 and m#2 were prepared with D_2O instead of H_2O and that may have improved the stability of GA.

Researchers who dilute GA-Multisol, GA-mixture #1, and GA-mixture #2 in saline have 3 h (Figure 3) or longer (Figures 4 & 5) before decomposition occurs. GA's stability in these dilutions is remarkable, and we suspect that propylene glycol is contributing to this stabilization in the presence of D_2O saline. A toxicity study has confirmed that GA-Multisol can be diluted from 1.9 mg/ml to 0.24 mg/ml with D_2O saline at room temperatures without loss of anticipated potency.[11]

In conclusion the vehicle modifications we have described may be helpful to researchers who are concerned with the potential toxicity of ethanol and the stability of dilute solutions of GA.

References

1. Chemical and Instrumental Verification of Organophosphorus Warfare Agents, Prepared for The Ministry for Foreign Affairs of Finland by The Advisory Board for Disarmament, Helsinki, 1977.
2. Larson L. The hydrolysis of dimethylamido-ethoxy-phosphoryl cyanide (Tabun), Acta Chim.Scand. 1953; 7: 306-314.
3. Hayes, TL, Shelf Life of Various Concentrations of XCSM in Diluents Under Selected Storage Conditions. Battelle Memorial Institute, Columbus, OH, Contract No. DAMD17-89-C-9050, ADP008825.
4. Physicians' Desk Reference, 56th edition, p 3026, Medical Economics Company, Montvale, NJ, 2002.
5. Capacio BR, Shih T-M. Anticonvulsant action of anticholinergic drugs in soman poisoning. Epilepsia 1991; 32: 604-615.
6. McDonough JH, Zoeffel, LD, McMonagle J, Copeland TL, Smith CD, Shih, T-M. Anticonvulsant treatment of nerve agent seizures: anticholinergics vs diazepam in soman-intoxicated guinea pigs. Epilepsy Research 2000; 38: 1-14.
7. McDonough JH, McMonagle J, Copeland T, Zoeffel D, Shih T-M. A comparative evaluation of benzodiazepines for control of soman-induced seizures. Archives of Toxicology 1999; 73:473-478.
8. Koplovitz, I., Schulz, S., Shutz, M., Railer, R., Macalalag, R., Schons, M., McDonough, J. Combination anticonvulsant treatment of soman-induced seizures. J. Appl. Toxicol. 21, S53-S55 (2001).
9. Joiner RL, Dill GS, Hobson DW, Olson CT, Feder PI, Menton RG, Kiser, RC, Task 87-35 Evaluating the efficacy of antidote drug combinations against soman or tabun toxicity in the rabbit. Battelle Memorial Institute, Columbus, OH, Contract No. DAMD17-83-C3129. AD A216386, 1989.
10. Logan, Thomas P., Allen, Edward D., Way, Mark R., Swift, Austin T., Soni, Sunil-Datta A method for the analysis of tabun in multisol using gas chromatographic flame photometric detection. Toxicology Mechanisms and Methods, 16:359-363, 2006.
11. Irwin Koplovitz, Personal Communication.